Experimental ischemia–reperfusion: biases and myths—the proximal vs. distal hypoxic tubular injury debate revisited

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Although the understanding of processes associated with hypoxic tubular cell injury has remarkably improved, controversies remain regarding the appropriateness of various animal models to the human syndrome of acute kidney injury (AKI). We herein compare available experimental models of hypoxic acute kidney damage, which differ both conceptually and morphologically in the distribution of tubular cell injury. Tubular segment types differ in their capacity to mount hypoxia-adaptive responses, mediated by hypoxia-inducible factors (HIFs), and in cell type–specific molecules shed into the urine, which may serve as early biomarkers for renal damage. These differences may be of value in the perception of the human AKI, its detection, and prevention.

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This review is in the memory of Dr Franklin H Epstein, a great physician-scientist, mentor, and friend, who first suggested the concept of medullary hypoxic damage in AKI.

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THE PROBLEM

Over the last four decades, the human situation of acute tubular necrosis/acute kidney injury (ATN/AKI) has been largely interpreted in terms of a single animal model, warm ischemia reflow, that is, a complete interruption of arterial blood flow for varying periods of time with maintenance of body temperature and then reflow. Many publications and oral presentations present the findings in this experimental model in the context of human AKI, failing to distinguish any differences. Although the drawbacks of this model have been underscored,[1–4] much of the myth of its equivalence to human AKI persists. A recently reviewed manuscript begins by the statement that 'Ischemia reperfusion injury is the major cause of AKI and occurs frequently due to the obligatory interruption of blood flow…leading to renal tubular necrosis, particularly in proximal tubule cells located in the outer medullary region…which are the most susceptible to injury and cell death.' This statement is entirely correct regarding the nature of AKI among rodents residing in renal research laboratories, as warm ischemia–reperfusion (WIR) is the most extensively used animal model, used in over 43% of AKI studies, published in *Kidney International* and in *NDT* over the last 3 years. Nevertheless, fundamental differences exist between WIR models and human ATN/AKI, calling for caution in implementing insights achieved by such models into clinical practice. Indeed, none of the many interventions that have proven effective in this setting alone have ever survived critical testing in the human situation.

The aim of this review is to examine WIR in the context of both its human functional and morphological equivalence and consequences. It will be compared with other hypoxic AKI models, namely cold ischemia and reperfusion (CIR) and the so-called 'distal nephron' models. We shall also discuss the implications of recent advances regarding tubular cell hypoxic stress response and biomarkers for tubular cell injury in the understanding of these AKI animal models.

THE HUMAN HYPOXIC AKI

Data regarding the pathophysiology and morphology of human AKI are limited. The clinical presentation is often

subtle, detected late, well into the established phase, associated with multiple co-predisposing factors, and without a clear-cut knowledge about the presence and distribution of renal parenchymal damage. It is important to realize that although renal tissue sampling is infrequent in this situation, there is a relative uniformity of opinion that overt tissue injury is limited. Nearly all pathologists recognize this and only make a diagnosis of AKI/ATN in clinical context, that is, 'basically intact renal morphology, compatible with AKI/ATN' (Figure 1a). This dyssynchrony of structure and function occurs in the transplant biopsy as well. On rare occasions, however, extensive injury is found (Figure 1b), usually after hemodynamic catastrophes, with protracted severe renal ischemia, such as after prolonged resuscitation, or post-partum severe hemorrhage.[4] Studies by Solez *et al.*,[5] and later by Olsen and Hansen,[6] show that tubular necrosis (as defined by cell loss; electron microscopic observations) in ATN/AKI is, indeed, usually very focal and limited, and seems to affect distal tubular medullary segments (medullary thick ascending limbs (TALs) and medullary collecting ducts (CDs)) to a larger extent than proximal tubular segments located in the outer medulla or the cortex.[6] Interestingly, these observations are in agreement with historical autopsies in the pre-dialysis era in patients dying with AKI. These reports, although imperfect to large extent, illustrate a focal and limited injury pattern, and underscore a predominantly distal nephron injury, named at that time 'lower nephron nephrosis.'[7]

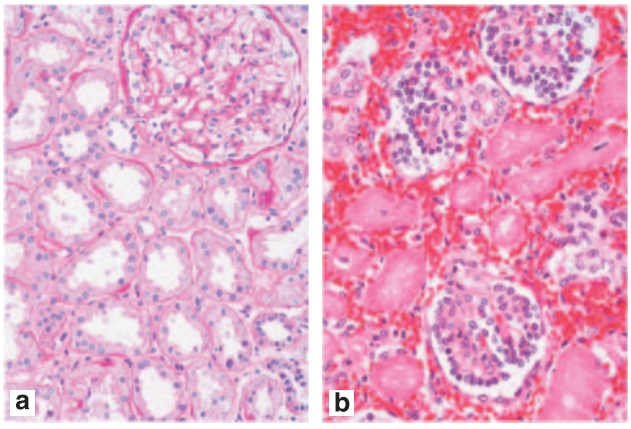

Figure 1 | Human hypoxic AKI: diverse morphology and function/morphology associations. (**a**) Kidney biopsy of a 25-year-old patient who presented with malaise and oliguric acute renal failure a few days after cocaine use. A near-normal morphology (other than some brush border diminishment, mild interstitial edema, and mononuclear infiltration) stands in sharp contrast with a rising serum creatinine of 7.5 mg/dl on the day of biopsy. This is a paradigm of human hypoxic AKI, characterized by normal cortical morphology obtained by biopsy (adapted with permission from Rosen and Stillman[4]). (**b**) Autopsy of a newborn with huge diaphragmatic hernia and cardiorespiratory failure despite life support management, succumbing 24 h after a Cesarean section to bradycardia followed by a complete loss of pulse for at least 20 min. Extensive proximal tubular necrosis is noted within the cortical labyrinth, with preservation of glomeruli and distal nephron elements. This is the morphologic and physiopathologic equivalent of the rat warm ischemia reflow model, with a good function/morphology correlation. As the human outer stripe is minimal, such ischemic changes would be manifested in the cortex. AKI, acute kidney injury.

This focal tubular injury includes the loss of brush border, and the finding of individual tubular cell loss by electron microscopic studies.[8–12] Indeed, intact tubular cells in urine sediments of patients with ATN are present and have been shown to be viable. The extrusion of such cells into the urine might lead to re-apposition of the tubular epithelium, such that the tubule appears uninjured. Recent studies show that tubular reconstitution can be achieved by the division of mature cells, and studies of cyclin D1 indicate that such a regenerative pool exists in human kidneys.[13] In some situations, apoptotic changes are also detected in human AKI, as shown in distal nephron segments in nephrotoxic ATN.[14] Distal tubular cell apoptosis in donor biopsies before engraftment was predictive of delayed graft function.[15] Kwon's[16] group has suggested that diminished nitric oxide generation by the injured endothelium and loss of the macula densa neuronal nitric oxide synthase may contribute to sustained AKI after engraftment. Aberrant hemodynamic regulation in this situation of delayed graft function might be related to morphological changes in vascular smooth muscle and endothelial cells.[17] The situation of 'ATN' in the transplant kidney is not exactly the same as native 'ATN,' but does provide us with analogous circumstance of morphological/functional dissociation and tissue is readily available to examine multiple parameters at specific time periods of well-documented kidney failure. The inflammatory component of human AKI/ATN is minimal and related to leukocytes within the vasa recta. Immunostaining of these cells indicated that some are hematopoietic cells[18] likely homed/transformed as a result of hypoxia, as it occurs in the areas of myocardial infarction scars.[19]

In summary, renal morphology of human AKI is dynamic, and it depends on the timing of sampling. Tubulointerstitial changes, if noted, are usually focal, encompassing tubular cell necrosis, as well as programmed cell death, sublethal forms of injury, and loss of viable cells. This may be accompanied with by regenerative changes, vascular injury, limited inflammation, tubular dilatation, and cast formation, principally in the established and recovery stages of ANT. Tubular injury involves various tubular segments, chiefly at the outer medullary region.

The limited and focal nature of human ATN markedly contrasts with a prominent decline in glomerular filtration rate (GFR), indicating a central role for altered glomerular hemodynamics, possibly related to a tubulo-glomerular feedback signal. In the absence of appropriate renal morphology and the limited clinical usefulness of timely determination of glomerular and tubular function, the need for new modalities identifying tubular damage and differing AKI from pre-renal azotemia is extremely important.

FEATURES OF THE WIR MODEL

In this technically simple model, renal blood flow is completely interrupted by clamping of the renal artery for

a determined period of time, usually at the range of 20–60 min. Throughout this period, the kidney is fully anoxic and nonfunctioning, that is, GFR and transport activity are fully abolished. ATN gradually develops upon reperfusion, its extent and consistency depending on the warm ischemia period. It is initiated at the corticomedullary junction in the outer stripe of the outer medulla, in the form of focal S3 proximal tubule (PT) damage, extending to the medullary ray and then finally involving the proximal convoluted tubules in the labyrinth. After 20–30 min of ischemia, S3 injury is limited and varied. As ischemic time progresses, injury becomes more consistent and confluent. After 45 min of ischemia, the outer stripe is extensively necrotic, accompanied by a variable degree of cortical necrosis (mainly proximal convoluted tubular segments in the labyrinth). After 60 min of ischemia, the degree of cortical necrosis approaches infarction.[20] Distal nephron injury (TAL) may develop if the ischemic period is prolonged or if upon reperfusion distal tubular oxygen consumption for transport is maintained or intensified.[21] Endothelial injury, manifested by outer medullary congestion, stasis, and hemorrhage, is accompanied by a substantial inflammatory response with the recruitment of neutrophils.[22] Renal dysfunction parallels the extent of tubular damage, with reduced GFR and sodium reabsorption, and all these injury markers are diminished by an extraordinary range of therapeutic interventions. GFR usually recovers within a week after WIR, but tissue damage and compromised renal microcirculation result in hypoxia-driven chronic progressive tubulointerstitial disease.[23,24] Initial tubular regeneration is extensive, derived from native undifferentiated cellular elements (S3) or from local stem cells (papilla) or exogenously derived (bone marrow).

DISSIMILARITIES OF WIR WITH HUMAN HYPOXIC AKI

WIR models markedly differ from human hypoxic ATN, both mechanistically and morphologically. Short-termed (20–40 min) total cessation of renal blood flow and transport activity is indeed encountered during living donor renal transplantation and in cross-clamped aortic/renal artery procedures. Nevertheless, most cases of 'hypoxic' AKI in clinical practice are characterized by compromised renal microcirculation and oxygenation that is related to systemic hemodynamic derangement, to sustained critical hypoxemia, or to various nephrotoxins that exert renal dysfunction to a large extent by compromising renal oxygenation (such as radiocontrast agents, nonsteroidal anti-inflammatory drugs, or amphotericin). In these settings, renal blood flow, though diminished at certain regions, never ceases, and oxygen consumption for residual tubular transport does persist. The implications of this fundamental difference are related to the variable dependency of tubular nephron segments on oxygen (reliance on aerobic oxidative metabolism by PT, as opposed to the capability to use anaerobic glycolysis by TALs and CDs) and the maintenance of energy stores in the presence of transport activity or in its absence.[25] While continuous supply of oxygen is obligatory for PT viability, TALs are quite resistant to hypoxia, as long as energy expenditure for transport activity is withheld.[26] This nephron segment shares with CDs a profound ambient physiological hypoxia, and can cope to some extent with anaerobic metabolism. However, transport activity is the major factor governing TAL injury during oxygen deprivation,[27] probably due to high mitochondrial respiration, leading to a rapid decline of energy stores and the formation of reactive oxygen species. Thus, with the complete cessation of GFR and tubular transport, induced by renal arterial clamping, although PTs are quickly affected, nonfunctioning TALs maintain viability.

A second major mechanistic dissimilarity with human hypoxic AKI is renal parenchymal temperature during ischemia, which is most often reduced throughout major cardiovascular surgery. Cadaveric transplants are kept at 4 °C during cold preservation, and even living-donor-transplanted kidneys are not kept at body temperature during the surgical procedure. Indeed, it is important to note that clamping of the renal artery results in an acute decrease in renal core temperature as long as the kidney is left exposed, as its warming is circulation-mediated. Reduction of renal temperature in these clinical scenarios is renoprotective. By contrast, in WIR models, the clamped kidney is most often placed back in place and is insulated, resulting in the maintenance of core temperature, so important to the full and consistent expression of structural damage.[28] Indeed, cold preservation with subsequent warm reperfusion (CIR) encountered in experimental transplantation leads to a better preservation of PTs, and a more pronounced damage to TALs upon reperfusion. In the original studies in transplanted rats, 12-h cold ischemia resulted in predominant TAL and inner medullary injury 24 h after transplantation, resembling distal nephron AKI models (see below). As the cold preservation time extends beyond 16 h, PT injury in the outer stripe and the cortex became evident as well.[29,30] Such findings are in agreement with personal observations (by S.R.) that features of both cold ischemia (inner stripe and papillary damage) and warm ischemia (outer stripe and cortical injury) occur if the cold ischemia time is prolonged. In a more recent report, 24-h cold ischemia followed by a shorter period (6 h) of transplant reperfusion, again, resulted in predominant TAL injury.[31] Finally, it is readily acknowledged that the outer stripe of the outer medulla, the region mostly affected by WIR, although highly developed in rodents, has limited representation in the human kidney.

Few additional clinically relevant hypoxic AKI models, also with predominant S3 injury, are characterized by profound and protracted renal hypoperfusion, produced by prolonged resuscitation,[32] with suprarenal critical aortic narrowing,[33,34] or by hemorrhagic shock.[35] It is conceivable that these models closely mimic the rare hemodynamic catastrophes in humans that end with extensive tubular injury as illustrated in Figure 1b.

FEATURES OF EXPERIMENTAL 'DISTAL NEPHRON' HYPOXIC AKI MODELS

As just stated, the rat kidney is anatomically different from the human kidney, but there is an extraordinary physiological similarity across mammalian species that concentrate urine. This ability is dependent on the counter-current system, in which the structure of the vasa recta (promoting reduced oxygenation by countercurrent oxygen shunting) and high level of sodium transport (oxygen consumption) predicate limited oxygen availability. Indeed, rats and humans may not share exactly the same renal anatomy, but it has been clearly shown that substantial hypoxia exists within the mammalian renal medulla under normal physiological conditions, a price paid for the ability to concentrate urine.[36]

This understanding has led to experimental 'distal nephron' models of ATN/AKI, based on the concept that in the physiologically hypoxic renal medulla, there is a delicate balance between oxygen supply and demand that maintains tubular cell viability. Hypoxic AKI represents disintegration of this equilibrium, caused by altered regional oxygen supply, enhanced oxygen demand for tubular transport, or their combination.[36] Isolated rat kidneys perfused with oxygenated, red-cell-free medium (i.e., profound hypoxia in a functioning kidney) develop outer medullary injury, principally affecting TALs in the mid-inner stripe of the outer medulla (as in Figure 2). S3 PT segments are involved as well, although to a lesser extent, evidently competing with TALs for the very limited ambient oxygen supply.[37] In addition to the corticomedullary gradient of hypoxic injury, an inner stripe damage gradient is also noted, related to the distance from the vasa recta, where tubules in the mid-interbundle zone, most remote from the vasa recta, are principally affected.[38] TAL injury pattern consists of mitochondrial swelling, with subsequent nuclear pyknosis and cell membrane fragmentation. Alterations of renal oxygenation and tubular transport, easily accomplished in the isolated kidney model, led to the recognition that TAL damage under hypoxic conditions correlates with transport activity. A nearly complete protection is achieved by transport inhibition, despite a significant decline in oxygenation and cellular energy stores,[27] suggesting a role for transport-associated mitochondrial membrane dysfunction in the development of cell injury, possibly due to the generation of reactive oxygen species.[39,40] Tubular damage is characteristically associated with altered sodium reabsorption. Most importantly, observations in the isolated kidney were extended to whole-animal models of hypoxic AKI, in which medullary oxygen balance was compromised by various combinations of insults, leading to enhanced single-nephron GFR and tubular transport, on one hand, and reduction of medullary blood flow, on the other hand. These insults included the blockade of prostaglandin and nitric oxide production (major factors in the maintenance of medullary oxygen balance), the administration of radiocontrast agents and vasoconstrictors, reduction in kidney mass (with hypertrophy of remnant nephrons), the induction of salt depletion, heart failure, diabetes, endotoxemia, myoglobinuria, urine outflow obstruction, or tubulo-interstitial disease.[24,36,41] In all these models, a similar pattern of predominant TAL injury is noted (Figure 2), with comparable distribution pattern, accompanied by S3 damage in the adjacent outer stripe. CDs are also affected, with overt papillary necrosis noted in some models. Additional PT

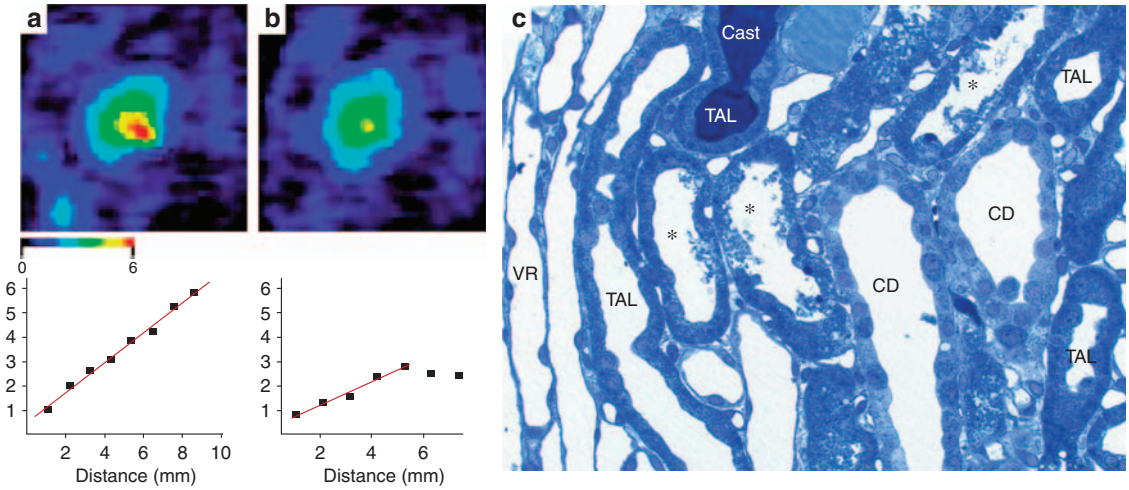

Figure 2 | Experimental distal tubular ATN in a rat hypoxic AKI model. Medullary hypoxic damage is induced by the administration of radiocontrast medium, concomitantly with the inhibition of prostaglandin and NO synthesis.[73] As compared with intact animal (as shown in **a**), in (**b**), tubular dysfunction is shown as early as 4 h after the induction of AKI with the use of sodium magnetic resonance imaging. The early fall in corticomedullary sodium gradient is the hallmark of evolving injury of the countercurrent urine concentration machinery. It parallels the development of isosthenuria, an early indicator of ATN in clinical practice. (**c**) Focal medullary thick ascending limb (mTAL) injury is already noted by that time, maximal in the mid-inner strip, where 4% of mTALs are affected. An injury gradient is seen, principally affecting tubules in the mid-interbundle zone (*), most remote from the vasa recta (VR), whereas mTALs adjacent to the VR are intact. This limited injury pattern is associated with variable renal dysfunction, with some 10–100% increments in plasma creatinine by 24 h (CD, collecting ducts; a and b adapted with permission from Maril *et al.*[61]).

changes are traced in specific models, such as PT vacuolization after radiocontrast, or S1–S2 PT injury after myoglobinuria.

In the most extreme models, the extent of damage that may reach 30–50% of TALs in the mid-inner stripe of the outer medulla correlates with renal dysfunction.[42] However, this association is lost in more moderate models, in which TAL injury average is about 10% or less. In these settings, rare focal tubular injury often stands in sharp contrast with a substantial decline in GFR, associated with preserved sodium reabsorption, conceivably reflecting a prominent role for altered glomerular hemodynamics. It is noteworthy that, an inverse correlation may exist between the extent of proximal (S1,S2) and distal tubular injury in complex sequential models. This may reflect an effect of declining GFR, caused by PT injury, on downstream distal tubular transport activity and damage.[43] By contrast, S3 and TAL hypoxic injury usually parallel in distal nephron models, conceivably as they both reside in the oxygen-deprived outer stripe and medullary rays.

Regeneration and cellular replacement in focal distal tubular damage is more likely achieved by cellular reapposition or division of mature cells, as opposed to the extensive and complex process noted in WIR.

SIMILARITIES AND DISSIMILARITIES OF HYPOXIC HUMAN AKI AND DISTAL NEPHRON MODELS

Human studies disclose physiological medullary hypoxia, as documented noninvasively using BOLD MRI (blood–oxygen level-dependent magnetic resonance imaging).[44] Furthermore, intensified medullary hypoxia has been detected in various settings that induce or predispose to hypoxic AKI, such as the administration of nonsteroidal anti-inflammatory drugs or radiocontrast agents,[45,46] in diabetes[47–49] or in patients with chronic kidney disease (Manotham K. *J Am Soc Nephrol* 2006; **17**: 164A). Conceivably, papillary necrosis noted in sickle cell disease, nonsteroidal anti-inflammatory drugs, and obstructive uropathies also reflects medullary hypoxic AKI.

AKI is most often encountered in patients with comorbidities that potentially predispose to renal hypoxia. Indeed, many of the animal models detailed above are designed to resemble such common clinical scenarios, leading to intensified medullary hypoxia and hypoxic AKI. It is important that, altering prostaglandin and nitric oxide synthesis recapitulates clinical conditions characterized by such altered systems, such as diabetes or aging.[36,50]

It is important that the extensive TAL injury noted in the cell-free perfused kidney or in the most severe distal nephron models is unlikely to occur in human ATN. The parallel to the human situation is far better represented by the more moderate models that have a more limited injury pattern.

HYPOXIA-ADAPTIVE RESPONSES IN AKI MODELS

Hypoxia invokes cellular hypoxia response, mediated to a large extent by hypoxia-inducible transcription factors (HIFs), leading to the activation of a host of genes, many of which are cell-protective.[36] HIF response is prompt and transient, principally regulated through the degradation of HIFα subunits by specific oxygen-sensitive prolyl hydroxylases. Tubular cells differ in their capacity to generate HIF. *In vivo*, CDs are potent HIF activators, whereas TALs are weak, and PT capacity to activate HIF is intermediate.[51,52] This might explain the marked tolerance of CDs to hypoxia in AKI models.

As partially illustrated in Figure 3c and f, all renal parenchymal cells are capable to express HIF after hypoxia or hypoxia mimetics such as HIF prolyl hydroxylase inhibitors. In both WIR and distal nephron AKI models, HIF activation occurs in the renal zones where tubular injury takes place: the outer stripe of the outer medulla (Figure 3b) and the inner stripe (Figure 3e) and inner medulla, respectively.[52]

Intensification of HIF response by prolyl hydroxylase inhibition has been suggested to confer resistance to various forms of AKI. Indeed, this approach or the administration of the HIF-mediated erythropoietin are the only maneuvers, so far, that were clearly shown to attenuate both PT[53–56] and TAL hypoxic injuries.[57,58] In addition to possible activation of comparable survival pathways in the two tubular cell types, this finding may underscore the importance of HIF-mediated protection of the renal microcirculation,[57] an inherent component of AKI shared by all models.

ASSESSMENT OF EXPERIMENTAL HYPOXIC TUBULAR DAMAGE: METHODOLOGICAL ADVANCES

Fine morphology in semi-thin sections of perfusion-fixed kidneys, supplemented by electron microscopy, is essential for proper assessment of experimental tubular damage, allowing optimal identification of tubular cell types and detection of various forms and stages of cell injury and death. This may be complemented by immunostaining and molecular imaging techniques for the detection of programmed cell death, disrupted cytoskeleton, and loss of cell polarity. These advanced methods enable the identification of an injury pattern that is apparently limited, or obscured by reparative processes.

Renal injury is a dynamic process and morphology at one time period provides only a limited perspective. Timing is therefore a crucial point, considering the remarkable plasticity of renal morphology that relates to both cell dropout/loss and reparative processes. By complementing morphology with immunostaining for both HIF and the hypoxia marker pimonidazole (which binds to tissue at pO_2 below 10 mm Hg, as shown in Figure 3), and killing animals at different time points, one can scan the extent and distribution of hypoxia, hypoxia cell response and cell damage, identifying simultaneously lethal as well as sublethal hypoxic cell injury. Indeed, HIF upregulation in morphologically unremarkable human allografts provides us with a marker for subtle/sublethal hypoxic injury.[59]

Evolving useful noninvasive modes detecting renal parenchymal stress include BOLD MRI, which maps intra-renal hypoxic regions,[44,45] or contrast MRI that detect altered

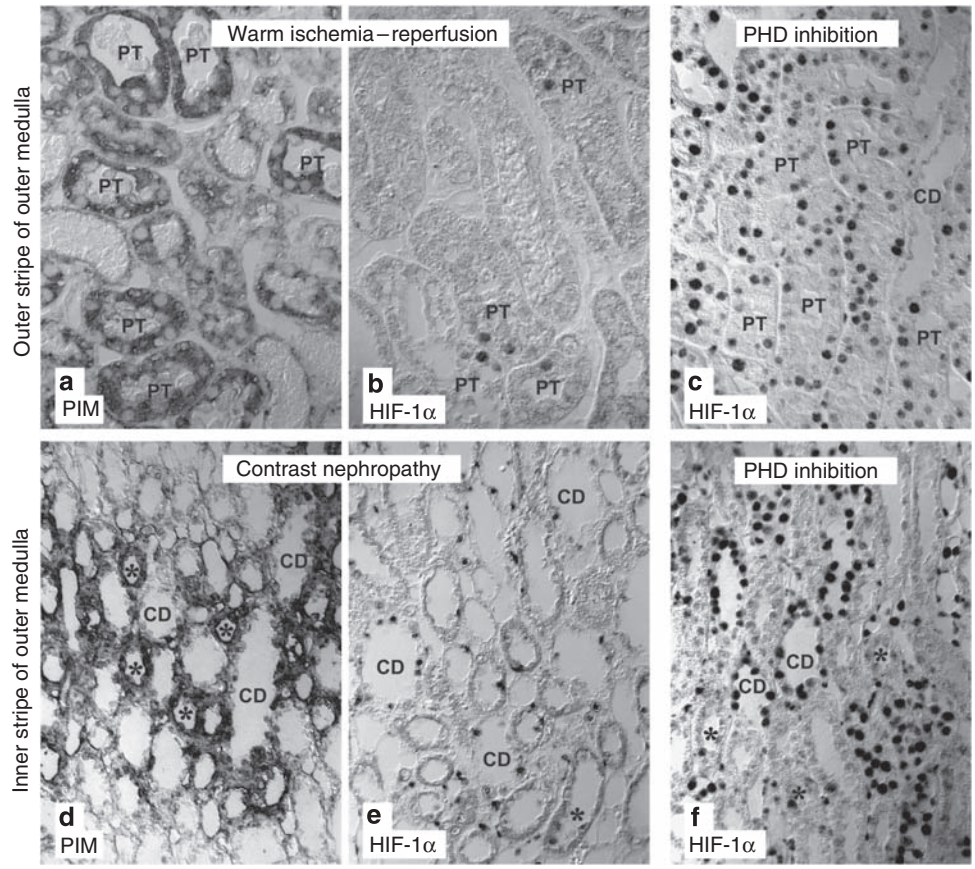

Figure 3 | Diverse patterns of hypoxia distribution and HIF response in hypoxic AKI models. At 24 h after insult, hypoxia (illustrated by pimonidazole adducts, PIM) is present in both WIR (45 min ischemia) and a distal nephron AKI model of contrast-induced nephropathy,[73] in the outer and inner stripes of the outer medulla, respectively (**a** and **d**). HIF responses also differ: minimal upregulation in the outer stripe in WIR (**b**) and a much more extensive increase in the contrast-induced nephropathy in the inner stripe (**e**) and in the papilla (data not shown). For comparison, the greatest stimulation of renal HIF occurs with chemical inhibition of HIF degradation (**c** and **f**),[57] underscoring a potential therapeutic implication of such a strategy. HIF, hypoxia-inducible transcription factor; WIR, warm ischemia–reperfusion.

perfusion and filtration.[60] Sodium MRI identifies functional derangement of medullary sodium gradient, reflecting loss of the countercurrent system (Figure 2), heralding evolving hypoxic distal nephron injury.[61]

Biomarkers are the most prominent clinically applicable breakthrough technologies in the early detection of AKI.[62] Interestingly, two most studied urinary biomarkers, kidney ischemia molecule (KIM)-1 and neutrophil gelatinase-associated lipocalin (NGAL), originate from PTs and TALs, respectively. Increased NGAL and KIM-1 in the urine is indeed noted in AKI patients, reflecting injury in both tubular segments.[63–68] It is tempting to assume that recovery of urine-cell-specific biomarkers may provide insight regarding the distribution pattern of tubular injury during hypoxic AKI. Urinary NGAL was found to be a most insightful marker of radiocontrast nephropathy,[69] a prototype of hypoxia-mediated nephrotoxicity with distal tubular injury. However, urine NGAL is also increased in experimental cisplatin toxicity, characterized by S3 injury,[70] suggesting that both proximal and distal tubular injuries coexist, possibly reflecting direct cell toxicity (S3), combined with regional hypoxic damage (S3 and TAL).

CLINICAL IMPLICATIONS

The differentiation between proximal and distal injury is not merely semantic, and may have profound therapeutic implications. For instance, enhancing GFR in evolving AKI has been adopted by most clinicians, anticipating a better outcome. However, according to the distal nephron injury concept, reduced GFR during AKI is an adaptive response, designed to reduce medullary oxygen demand, restoring medullary oxygen balance. In this perspective, increasing GFR might intensify medullary hypoxia and distal tubular damage. Indeed, studies designed to enhance GFR in AKI were not effective, and were even deleterious in subsets of patients.[71] On the other hand, attenuation of tubular transport with the use of loop diuretics, providing improvement of medullary oxygenation, a reasonable strategy according to the same concept, has not been successful as well, and has even shown deleterious effect following radiocontrast studies.[72] A major caveat in many of these clinical trials is that therapeutic interventions were initiated based on rising plasma creatinine, well into the established phase of AKI. It is anticipated that the evolving field of urinary biomarkers will enable an early detection of AKI and

intervention at its initiation phase. Furthermore, simultaneous detection of changes of both specific distal and proximal urinary biomarkers might help in designing therapeutic interventions, according to the relative importance of each tubular segment in different AKI subtypes.

In conclusion, in human hypoxic AKI, focal and limited tubular cell damage sharply contrasts with severe impairment of renal function. Unfortunately, this phenomenon is poorly depicted by most experimental models, eliciting extensive injury. Therefore, the clinical relevance of such models, although highly consistent, is limited, and their pattern of injury distribution may be misleading. Both proximal and distal tubular hypoxic injuries may be observed under diverse clinical conditions, possibly with dissimilar therapeutic implications. Evolving strategies targeting renal microcirculation, hypoxia response, and urine biomarkers could form a base for a better detection and characterization of human hypoxic AKI. This could lead to more appropriate and hopefully effective interventional strategies.

DISCLOSURE

All the authors declared no competing interests.

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